Adjustable Pinhole, in particular for a Laser Scanning Microscope

The geometrical parameters of the pinhole are decisive for the efficiency of the laser scan microscope. The smaller the pinholes are and the more precisely they can be adjusted, the higher the resolutions of the laser scan microscope that can be achieved and, accordingly the smaller the dimensions of the microscope optics that are possible. It is advantageous, if the pinhole apertures can be adjusted to sizes of 3μm or greater. In this case, the reproducibility of 0.3μm in the adjustment of the pinhole aperture and position is essential. Still smaller pinhole apertures lead to high intensity losses due to diffraction of the light passing through the edge area of the pinhole. The pinhole aperture, which is mainly arranged in front of the photoreceiver, need not be circular but can also have a square form.

For the adjustable pinholes in laser scan microscopes, elements are used, which have, preferably in order to give a square aperture, adjustable straight physical edges. In order that these edges are as sharp as possible and the aperture has a slight extension in the direction of the light flux as a result, these elements are either fastened at the edges of the aperture or the elements are thin foils. The state-of-the-art of the finishing technology enables the making of edges, which have pinholes with extension greater than 10μm in the direction of the light flux. However, in such pinholes, there is the danger of vignetting of the light, if the size of the aperture is to be adjusted to less than 10μm.

Thus, in DE 202 05 079 U1, a variable pinhole for a confocal scan microscope is described, which consists of two diametrically movable aperture claws with grooves. This type of groove can be produced with sharp edges only to a limited extent, therefore such pinholes have an extension of about 100μm in the direction of the light flux. The disadvantages of this solution lie in the danger of the vignetting of the light and the unavoidable curves at the corners in the grooves. Both lead to the consequence that the square-shaped apertures cannot be adjusted to sizes smaller than 10μm. Sufficiently small oblique positioning of the pinhole, with less than 0.1°, for instance, can be achieved only with relatively high technical expense.

The aim of the invention is to realize an adjustable pinhole, which enables apertures with sizes greater than 3μm in a field of $1 \times 1 mm^2$ with the tolerance of 0.3μm. Thereby, it is important to keep the physical dimensions of the aperture in the direction of the light flux as small as possible, for example smaller than 10μm, in order to avoid the vignetting of the light passing through the aperture.

The proposed solution lies in using two mirror-inverted and mutually displaceably arranged silicon apertures for the pinhole in the laser scan microscope, each with a square etched window.

The advantage of the solution lies in that the silicon has a cubic crystalline structure, and therefore square apertures, whose edges are atomically sharp-edged, are generated in the photolithographic etching. Furthermore, the flanks of the aperture exhibit an acute-angled etching angle of 54.7°, so that the mirror-inverted arrangement of two silicon aperture pinholes, with arbitrarily small openings and arbitrarily small extent in the direction of the light flux can be built.

A further advantage lies in that, with silicon as the material for the aperture, optically soft apertures are built within a certain visual spectral range of about 500nm, because silicon is increasingly transparent to light with increasing wavelength in that spectral range. As a result, within this limited wavelength range of about 500nm, diffraction effects at the aperture can be avoided or reduced. This leads to reduction in the loss of the light in the laser scan microscope due to the diffraction and to higher light intensities on the photoreceiver for small pinhole apertures and hence to greater sensitivity of the laser scan microscope in this spectral range of light. The disadvantage of silicon, namely its increasing light transparency in the region of the visual red onwards, can be eliminated by means of a reflection or an absorption coating on its surface.

Figure 1: Pinhole with 2 silicon apertures

In silicon plates with outside dimensions of about 7x5x0.5 mm^2, windows of size 1x1mm^2 are etched photolithographically. The silicon apertures are arranged mutually pairwise, so that the etched windows lie mutually mirror-inverted. The fastening of the silicon apertures on the mechanical contrivance of the pinhole is done by gluing. A distance plate serves the purpose of retaining the necessary distance between the upper aperture and the mechanical contrivance of the pinhole. A spacer foil, which is arranged between the apertures, enables the achieving of a pinhole height of 10μm. By means of an antiparallel movement of both the elements of the mechanical contrivance of the pinhole, the pinhole size can be adjusted between 0 and 1mm.

The direction of the movement of the mechanical contrivance of the pinhole is thereby antiparallel to the diagonal of the etching window in the silicon plate.

A protective coating is provided on the Si aperture in order to hinder the passage of the light through the aperture outside the opening because silicon is increasingly transparent to light with higher wavelength for wavelengths greater than 500nm. For this, a layer of chromium, gold, aluminum or silver of about 100nm thickness can be vapor deposited on the silicon apertures. All these layers have, however, the disadvantage of high reflectivity, so that interfering stray light scattered due to the reflection is generated in the laser scan microscope.

More suitable are black light absorbing protective layers, such as those that can be coated, for example, by means of special vapor deposition.
Contrary to expectations, one can also work without the coating, which is advantageous in wavelength range up to 600nm.
For the justification, the adjustment of the openings with a small aperture, for example with a size of 10µm, is done under a microscope by means of manipulators. Since, due to the small height of the pinhole, a depth of field of only 10µm is necessary for the microscope, it is possible to use high resolution microscopes with an aperture of, for example, A=0.8. In the common types of pinholes, this is not possible because the microscope must have, due to the pinhole height of 100µm, a correspondingly high depth of field, and a small aperture of, for instance, A=0.1.

Figure 2: Justification of the silicon apertures by means of integrated motor drives

Elements 1 and 2 are displaceable antiparallel in the x-direction and support both the silicon apertures, whereby at least one aperture is supported on another element 3, which is displaceable perpendicular to the direction of the displacement of the elements 1 and 2.
Element 3 is fixed on two flexible solid joints, which exhibit high rigidity in the direction of the displacement of the elements 1 and 2 and exhibit high flexibility in the direction of the displacement of the element 3.
The displacement of the element 3 in the y-direction takes place by means of two spindles driven by a motor, whereby the motor is arranged in a rotation-proof and displaceable manner on element 2. The two spindles, each one of which grips a screw nut fastened on elements 2 and 3, exhibit slopes that are advantageously different, for example, there can be a slope difference of 50µm.

The silicon apertures are glued in a pre-justifiable position on the elements 1 and 3 of the mechanical contrivance of the pinhole (**Figure 2**). Due to the motor drive with difference threaded spindles acting between the elements 1 and 3 in the y-direction, and due to the arrangement of the joints (for example, solid parallel spring joint), the justification, as well as the later readjustment of the silicon apertures with resolutions of, for example, 0.1µm, is possible in the laser scan microscope at any time. The resolution of 0.1 µm follows from the slope

difference of the threaded spindles of, for instance, 50µm, from the microstep operation of the stepmotor with 16 microsteps per full step and from 20 full steps per motor revolution. In the justification, the elements 1 and 2 are adjusted antiparallel to the x-direction, and the element 3 is adjusted in the y-direction by means of the motor until a square pinhole of, for instance, 10x10 mm^2 is formed. The pinhole aperture is now square with that size, as it will be for other sizes, if the elements 1 and 2 are displaced antiparallel in the x-direction. The advantage is that, in this manner the pinhole of the laser scan microscope can also be readjusted afterwards anytime, if its microscopic observation there is possible. The elements 1 and 2 are moved antiparallel in the x-direction for the adjustment of the pinhole size by means of a scissors-like mechanism not shown here.

The pinhole can be adjusted to a square form in the laser microscope at any time, even without its microscopic observation, solely on the basis of the evaluation of the photoreceiver signals of the laser scan microscope. For this purpose, the elements 1, 2 and 3 are adjusted by means of a motor-driven scanning process in such a manner that, with the pinhole aperture as small as possible, the light falling on the photoreceiver has the maximum intensity. In that case, the pinhole must have a square form. For this purpose, with the stepwise reduction in the size of the opening of the aperture (x-drive), the Si aperture on the element 3 is adjusted by means of the y-drive in such a manner that the photoreceiver receives the maximum light signal. This scanning process is repeated alternately with the x- and y-drives, until the maximum light signal of the photoreceiver reaches a prespecified minimal value, which corresponds to the pinhole size of, for instance, 10µm. Following this automatic justification process, the pinhole aperture has necessarily a square form for all sizes.

Figure 3: Pinhole area in dependence of the pinhole dejustification y_{just}

As it can be seen from the family of curves in Figure 3a, due to the quadratic dependence of the pinhole area with respect to the pinhole dejustification for $y_{just} = 0$, one obtains a very low sensitivity of the photoreceiver with respect to the adjustment in the y-direction and the dejustification at $y_{just} = b$. Therefore, the flank method, in which, during the scanning in the y-direction, on each of the two symmetrical flanks, one measurement point is determined with high sensitivity and the square pinhole form is obtained by adjusting to the average value for the two flanks, can be employed here advantageously.

This justification is initiated with a relatively large pinhole aperture of, for example, 100µm, and is concluded with a pinhole size that is as small as possible, for example, 10µm. The result for all pinhole sizes is necessarily a square pinhole aperture.

Exemplary values are given in Figure 3b,